BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

MERRILL
CORPORATION
CORPORATE, LEGAL, FINANCIAL PRINTING
225 VARICK STREET
NEW YORK, NY 10014 • PHONE: (877) 466-8398

MERRILL TRANSLATIONS

State of New York)
) ss:
County of New York)

Certificate of Accuracy

This is to certify that the attached document, Chinese Patent Application 98112264.7, originally written in Chinese, is, to the best of our knowledge and belief, a true, accurate and complete translation into English.

Dated: November 3, 2003

Kara M. Dempsey
Vice President
Merrill Corporation

Sworn to and signed before
Me this 3rd day of
November, 2003

Notary Public

ANNE S. HASKELL
Notary Public, State of New York
No. 02HA5033933
Qualified in New York County
Commission Expires 10/27/2005

[19] National Intellectual Property Rights Office, People's Republic of China [51] Int.Cl[7]
C12P 21/00
C07K 19/00

[12] Invention Patent Application Disclosure and Specifications

[21] Application no. 98112264.7

[43] Disclosure date March 29, 2000 [11] Disclosure no. CN 1248631A

[22] Application date September 24, 1998
[21] Application no. 98112264.7
[71] Applicant: Guoqing Zhou
Address Sichuan Yufeng Machine Factory Zhoushucheng, Rongxian, Sichuan Province 643100
[72] Inventor: Guoqing Zhou
Patent Claims Document 1 page, Specifications 2 pages, Attached Figures 1 page

[54] Name of invention: A fusion protein for immunoprophylaxis and immunotherapy of venereal disease and cancer.

[57] Abstract

The present invention relates to fusing the bacteria *mycobacterium bovis var. BCG* (M. Bovis BCG) heat shock protein to a tumor-specific antigen, i.e., the human papilloma virus (HPV), to form a fusion protein. This fusion protein can be expressed in E. coli, yeast and plants. Immunization using this fusion protein leads to a specific cellular immunity and humoral immunity against tumor-specific antigens. It not only protects against the human papilloma virus infection but also serves as immunoprophylaxis and immunotherapy for acute condyloma, tumors and cancer caused by HPV. Thus, the recombinant fusion protein can be used to prepare an effective prophylactic and therapeutic immunizing composition.

Published by the Patent Literature Publishing Company

ISSN1008-4274

1. The present invention relates to a fusion protein for treating venereal disease and cancer caused by venereal disease and a method of making a recombinant fusion protein by fusing a *mycobacterium bovis var. BCG* heat shock protein to a human papilloma virus (HPV) virus protein.

2. A method as described in Patent Claim 1, wherein a part or all of the heat shock protein is fused to a part or all of the human papilloma virus' virus protein to form a fusion protein.

3. A method as described in Patent Claims 1 and 2, wherein the N-terminal amino acids of the recombinant fusion protein come from *mycobacterium bovis var. BCG*'s heat shock protein, the C-terminal amino acids come from human papilloma virus (HPV) type 16 (HPV-16) or type 18 (HPV-18) early expression proteins (for example, HPV-16 E4, E5, E6 or E7).

4. A method as described in Patent Claims 1 and 2, wherein the N-terminal amino acids of the recombinant fusion protein come from human papilloma virus (HPV) type 16 (HPV-16) or type 18 (HPV-18) early expression protein (for example, HPV-16 E4, E5, E6 or E7), and the C-terminal amino acids come from *mycobacterium bovis var. BCG* heat shock protein.

5. A fusion protein as described in Patent Claims 1, 2, 3 and 4, wherein the N-terminus of the recombinant fusion protein can be linked to a multiple-histidine tag, to facilitate purification.

6. A recombinant fusion protein as described in Patent Claims 1, 2, 3, 4 and 5, which can be expressed via a plasmid vector in E. coli, yeast and plants.

7. A recombinant fusion protein as described in Patent Claims 1, 2, 3 and 5, wherein the N-terminal amino acids come from *mycobacterium bovis var. BCG*'s 65kDa or 70kDa heat shock protein, the C-terminal amino acids come from human papilloma virus (HPV) type 16 (HPV-16) or type 18 (HPV-18) early expression protein (for example, HPV-16 E4, E5, E6 or E7).

8. A recombinant fusion protein Hsp-E7 as described in Patent Claim 7, wherein the N-terminal 539 amino acids come from *mycobacterium bovis var. BCG* 65kDa heat shock protein and the C-terminal 98 amino acids come from human papilloma virus (HPV) type 16 (HPV-16) early expression protein E7.

9. A recombinant fusion protein Hsp-E7 as described in Patent Claim 8, wherein the amino acid sequence of the protein is shown in Specifications Attachment 1.

10. A recombinant fusion protein Hsp-E7 as described in Patent Claims 8 and 9, wherein the N-terminal of the protein can be linked to a multiple histidine tag, to facilitate purification.

11. A recombinant fusion protein Hsp-E7 or a recombinant fusion protein that contains multiple histidines at its N-terminus ((h)Hsp-E7) as described in Patent Claims 8, 9 and 10, said recombinant fusion protein not only possesses immunoprophylactic ability against human papilloma virus infection (HPV), it also possesses immunoprophylactic and immuno-therapeutic activity against acute condyloma, tumors and cancer caused by the human papilloma virus (HPV).

[handwritten] Implementation scenarios
(1) Recombinant fusion protein structure.

To form a recombinant fusion protein, a heat shock protein (Hsp) is fused to a tumor-specific antigen (HPV). Taking recombinant fusion protein Hsp-E7 for an example, the N-terminal 539 amino acids come from *mycobacterium bovis var. BCG* 65kda (kilo Daltons) heat shock protein and the C-terminal 98 amino acids come from early protein E7 of human papilloma virus (HPV) type 16. The protein's amino acid sequence is shown in Figure 1 and its molecular weight is approximately 67737 Daltons.

The N-terminus of HspE7can be linked to a multiple-histidine tag to facilitate purification.

(2) Recombinant fusion protein HspE7 expression in E. coli.

The DNA encoding a recombinant fusion protein is cloned into the pet28a carrier, which contains a T7 promoter. The expression of the protein is induced by IPTG. The DNA encoding the recombinant fusion protein can also be cloned into other E coli vectors and expressed in E. coli.

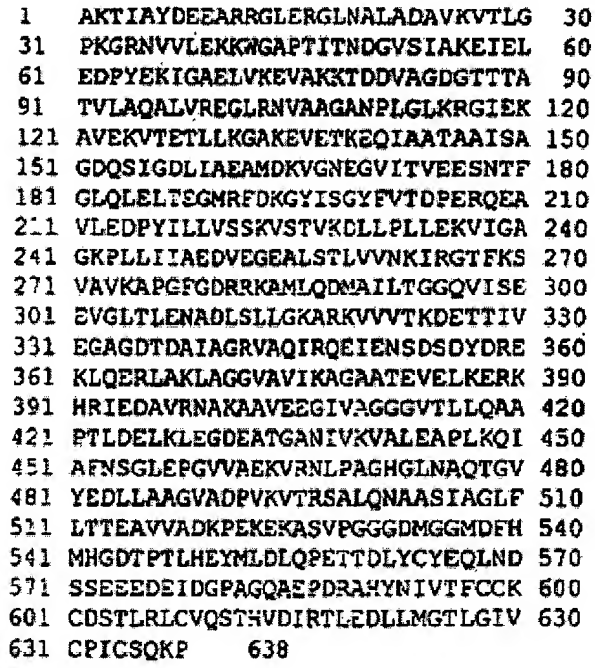
(3) Recombinant fusion protein expression in plants.

The DNA encoding a recombinant fusion protein can be cloned into pBI121 or pBI221, respectively. The resultant vectors can be introduced into plants using an agricultural bacterium and or a gene gun to express the protein. The DNA encoding the recombinant fusion protein can also be cloned onto other plasmid vectors and expressed in plants.

(4) The DNA encoding the above-mentioned recombinant fusion protein can be cloned into a yeast plasmid vector s, and expressed in yeast.

[handwritten] Implementation examples
(1) The DNA that encodes the recombinant fusion protein Hsp-E7 is cloned into a pet28a vector and is operatively linked to a T7 promoter. The protein is expressed in E. coli.
(2) The DNA that encodes the recombinant fusion protein can be cloned into a pBI121 or pBI122 vector and expressed in plants. Its promoter is 2xCaMV355 + AMV RNA4 promoter and enhancer
(3) The DNA that encodes the recombinant fusion protein can be cloned into a yeast plasmid vector and expressed in yeast.
(4) Using recombinant fusion protein Hsp-E7 to immunize mice not only induced cellular immune response, but also induced humoral immune response. These immune responses are specific to HPV-16-E7. In addition, immunization using these recombinant fusion proteins (in physiological saline) protects against HPV and tumor infiltration, and therapeutic immunization leads to elimination of existing tumors. Although, most of the data are obtained using the multiple-histidine-tagged recombinant fusion protein (h)Hsp-E7, direct comparison of it and the histidine-tag free recombinant fusion protein Hsp-E7 indicates that the two are similar in their ability to prevent HPV infection and eliminate acute condyloma and tumors.

Attachment 1.



[19]中华人民共和国国家知识产权局

[51]Int.Cl⁷
C12P 21/00
C07K 19/00

[12]发明专利申请公开说明书

[21]申请号 98112264.7

[43]公开日 2000年3月29日

[11]公开号 CN 1248631A

[22]申请日 1998.9.24 [21]申请号 98112264.7
[71]申请人 周国庆
地址 643100 四川省荣县四川格丰机械厂周树成
[72]发明人 周国庆

权利要求书 1 页 说明书 2 页 附图页数 1 页

[54]发明名称 一种免疫预防和免疫治疗性病和癌症的融合蛋白

[57]摘要

本发明融合细菌 myoobacterium bovis var. BCG (M. Bovis BCG)的热休克蛋白到 肿瘤特异性的抗原即人类乳头瘤病毒(HPV)上而构成融合蛋白。这种融合 蛋白表达在大肠杆菌、酵母菌和植物中。用这种融合蛋白进行免疫注射,产生 对肿瘤特异性抗原的特异性细胞免疫和体液免疫反应,不仅具有免疫预防人类 乳头瘤病毒(HPV)侵染的能力,而且,免疫预防和免疫治疗由 HPV 引起 的尖锐湿疣、肿瘤和癌症,因此,该种类型的重组融合蛋白,提供了一种简单 有效的预防免疫制剂和治疗免疫制剂。

ISSN1008-4274

1.本发明是一种治疗性病和由性病引起的癌症的融合蛋白。权利要求重组融合蛋白的方法，即融合 *mycobacterium bovis var. BCG* 的热休克蛋白(Heat shock protein)到人类乳头瘤病毒(HPV)的病毒蛋白上。

2.如权利要求 1 所述的方法，热休克蛋白的一部份或全部融合于人类乳头瘤病毒的病毒蛋白的一部份或全部上，构成融合蛋白。

3.如权利要求 1 、 2 所述的方法，重组融合蛋白 N-末端的氨基酸来自于 *mycobacterium bovis var. BCG* 的热休克蛋白， C-末端氨基酸来自于人类乳头瘤病毒（ HPV ）类型 16(HPV-16)或类型 18(HPV-18)的早期表达的蛋白(如 HPV-16 的 E4 或 E5 或 E6 或 E7)。

4.如权利要求 1 、 2 所述的方法，重组融合蛋白 N-末端的氨基酸来自于人类乳头瘤病毒（ HPV ）类型 16(HPV-16)或类型 18(HPV-18)的早期表达的蛋白(如 HPV-16 的 E4 或 E5 或 E6 或 E7)， C-末端氨基酸来自于 *mycobacterium bovis var. BCG* 的热休克蛋白。

5.如权利要求 1 、 2 、 3 和 4 所述的融合蛋白，重组融合蛋白的 N-末端可接多个组氨酸(Histidine)的领头顺序(Histidine-tagged)，便于纯化。

6.如权利要求 1 、 2 、 3 、 4 、 5 所述的重组融合蛋白，可通过质粒载体表达在大肠杆菌、酵母菌和植物中。

7.如权利要求 1 、 2 、 3 和 5 所述的重组融合蛋白，其 N-末端的氨基酸来自于 *mycobacterium bovis var. BCG* 的 65kDa 或 70kDa 热休克蛋白， C-末端氨基酸来自于人类乳头瘤病毒（ HPV ）类型 16(HPV-16)或类型 18(HPV-18)的早期表达的蛋白(如 HPV-16 的 E4 或 E5 或 E6 或 E7)。

8.如权利要求 7 所述的重组融合蛋白 Hsp-E7 ，其 N-末端 539 个的氨基酸来自于 *mycobacterium bovis var. BCG* 的 65kDa 热休克蛋白， C-末端 98 个氨基酸来自于人类乳头瘤病毒（ HPV ）类型 16(HPV-16)的早期表达的蛋白 E7 。

9.如权利要求 8 所述的重组融合蛋白 Hsp-E7 ，其氨基酸顺序如说明书附图 1 。

10.如权利要求 8 、 9 所述的重组融合蛋白 Hsp-E7 ，其 N-末端可接多个组氨酸（ Histidine ）的领头顺序，便于纯化。

11.如权利要求 8 、 9 、 10 所述的重组融合蛋白 Hsp-E7 和 N-末端可带多个组氨酸(Histidine)的重组融合蛋白(h)Hsp-E7 ，不仅具有免疫预防人类乳头瘤病毒（ HPV ）侵染的能力，而且具有免疫预防和免疫治疗由人类乳头瘤病毒(HPV)引起的尖锐湿疣、肿瘤和癌症的作用。

实施方案

(1)重组融合蛋白的结构

融合热休克蛋白(Hsp)到肿瘤特异性的抗原（ HPV ）上而构成融合蛋白。以重组融合蛋白 Hsp-E7 为例，其 N-末端的 539 个氨基酸来自于 *mycobacterium bovis var. BCG* 的 65kda(千道尔顿)的热休克蛋白； C-末端 98 个氨基酸来自于人类乳头瘤病（ HPV ）类型 16 的早期蛋白 E7，其蛋白质的氨基顺序如图 1，预计的分子量约 67737 道尔顿。

HspE7 的 N-末端可接多个组氨酸（ Histidine ）的领头顺序，便于纯化。

(2)重组融合蛋白 HspE7 在大肠杆菌中的表达。

重组融合蛋白克隆在 pet28a 载体上，其起动子为 T7，可被 IPTG 诱导表达该重组蛋白。重组融合蛋白也可克隆在其它大肠杆菌的载体上，表达在大肠杆菌中。

(3)重组融合蛋白表达于植物中

重组融合蛋白可克隆于 pBI121 或 pBI221 上，分别通过农杆菌和基因枪转入植物中，在植物中表达。重组融合蛋白也可克隆在其它质粒载体上，表达在植物中。

(4)重组融合蛋白可克隆于酵母的质粒载体上，在酵母中表达。

实施例：

(1)重组融合蛋白 Hsp-E7 克隆在 pet28a 载体上，其起动子为 T7，在大肠杆菌中表达。

(2)重组融合蛋白可克隆于 pBI121 或 pBI221 上，在植物中表达。其起动子为 2x CaMV35S+AMV RNA4 起动子和增强子。

(3)重组融合蛋白可克隆于酵母的质粒载体上，在酵母中表达。

(4)用重组融合蛋白 Hsp-E7 免疫老鼠，不仅诱导细胞免疫而且也诱导体液免疫。这些免疫对 HPV-16-E7 是特异性的。进一步， 用这种在生理盐水中的重组融合蛋白进行预防免疫，保护免受 HPV 和肿瘤的侵染挑战；而治疗性的免疫导致已存在的肿瘤消失。尽管大多数的资料是用一个具多个组氨酸的领头顺序的(Histidine-tagged)重组融合蛋白(h)Hsp-E7 得到的，但直接比较重组融合蛋白(h)Hsp-E7 与重组但没有组氨酸领头顺序(Histidine-tag)的融合蛋白 Hsp-E7，二者都具有同等预防 HPV 侵染、消除尖锐湿疣和肿瘤的能力。

98.10.13

说 明 书 附 图

图 1 .